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Abdul Malik <abdulmalik@unm.ac.id>

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**Dr. Alexey A. Maslakov** <no-reply@subs.elpub.ru> Reply-To: "Dr. Alexey A. Maslakov" <ges-journal@geogr.msu.ru> To: Abdul Malik <abdulmalik@unm.ac.id> Thu, Sep 29, 2022 at 7:18 PM

Dear Abdul Malik!

We have received the following manuscript to be considered for publication in the Geography, Environment, Sustainability journal:

# "CO2 UPTAKE IN ARTIFICIAL AND NATURAL MANGROVE FORESTS OF SOUTH VIETNAM"

### Abstract

Mangrove forests are one of the most productive and efficient long-term carbon sinks. Mangroves have experienced large-scale deforestation and conversion to other land uses, particularly in Southeast Asia. Present time the conservation of mangrove carbon stocks has been promoted in global climate negotiations due to their potential contribution to mitigating GHG emissions. However, uncertainty of estimating of CO2 fluxes remains recently due to geographical variability of mangrove forests and field data limitations. The paper presents the results of photosynthesis studies at the leaf level in-situ of seedlings of Rhizophora apiculata Blume, 1827 of natural and artificial origin. The research was carried out in a mangrove plantation located in the Can Gio Biosphere Reserve, located 50 km from Ho Chi Minh City (South Vietnam). The photosynthesis CO2 uptake was measured using a Portable Photosynthesis System LI-6800 (Li-Cor Inc., USA). The photosynthetic radiation is determining factor influencing the photosynthesis of the investigated seedlings of R. apiculata. Artificial seedlings growing in an open area had higher productivity and better photosynthetic performance. It was found that the obtained values of photosynthesis are distributed in three clearly marked zones, corresponding to the values of photosynthesis obtained in the pre-noon, noon and afternoon. The main inhibitory factor affecting the photosynthesis of R. apiculata is the disturbance of the water balance of the leaves. The optimum air temperature for the processes of photosynthesis in seedlings is (35 ± 2) °C. With an increase in the concentration of CO2 in the air, the intensity of photosynthesis also increases.

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## Dear Abdul Malik!

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Thu, Oct 20, 2022 at 9:27 PM

#### Research paper CO<sub>2</sub> UPTAKE IN ARTIFICIAL AND NATURAL MANGROVE FORESTS OF SOUTH VIETNAM

Nikolay G. Zhirenko<sup>1,2</sup>, Van Thinh Nguyen<sup>2</sup>, Juliya A. Kurbatova<sup>1,\*</sup> <sup>1</sup> A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia <sup>2</sup> Joint Russian - Vietnamese Tropical Scientific Research and Technological Center, Southern Branch, Ho Chi Minh <u>eityCity</u>, Vietnam

\* Corresponding author: kurbatova.j@gmail.com

ABSTRACT. Mangrove forests are one of the most productive and efficient long-term carbon sinks. Mangroves have experienced large-scale deforestation and conversion to other land uses, particularly in Southeast Asia. Present timeCurrently, the conservation of mangrove carbon stocks has been promoted in global climate negotiations due to their potential contribution to mitigating GHG emissions. However, the uncertainty of estimating of CO2 fluxes remains recently due to the geographical variability of mangrove forests and field data limitations. The paper presents the results of photosynthesis studies at the leaf level in-situ of seedlings of Rhizophora apiculata Blume, 1827 of natural and artificial origin. The research was carried out in a mangrove plantation located in the Can Gio Biosphere Reserve, located 50 km from Ho Chi Minh City (South Vietnam). The photosynthesis CO<sub>2</sub> uptake was measured using a Portable Photosynthesis System LI-6800 (Li-Cor Inc., USA). The pPhotosynthetic radiation is determining factor influencing the photosynthesis of the investigated seedlings of R. apiculata. Artificial seedlings growing in an open area had higher productivity and better photosynthetic performance. It was found that the obtained values of photosynthesiphotosynthesis values are distributed in three clearly marked zones, corresponding to the values of photosynthesis obtained in the pre-noon, noon, and afternoon. The reserves of waterwater reserves consumed in the midday time did not fully recover from the seedlings in the afternoon. The main inhibitory factor affecting the photosynthesis of R. apiculata (if we do not take into account PAR) is the disturbance of the water balance of the leaves. The optimum air temperature for the processes of photosynthesis in seedlings is  $(35 \pm 2)$  °C. With an increase in the concentration of CO2 in the air, the intensity of photosynthesis also increases.

**KEYWORDS:** *Rhizophora apiculata*, air temperature, CO<sub>2</sub> concentration, diurnal dynamics, intensity of photosynthesis, light response curve

#### CONFLICTS OF INTEREST

The authors reported no potential conflicts of interest.

#### INTRODUCTION

Mangrove forests are one of the unique forest ecosystems. They are an important part of tropical coastal ecosystems. Being of great ecological importance, mangroves also fulfill protective and economic functions (Donato et al. 2011; Hogarth 2007; Hogarth 2008; Simard et al. 2019; Saintilan et al. 2020).

However, the degradation of mangrove ecosystems is currently being observed (Alongi 2002; Valiela et al. 2001; Nguyen 2000). This is due both to economic activity, which mainly consists of the deforestation of these forests (Luong 2014) and to the ongoing global warming (Desherevskaya et al. 2013), which results in both sea-level rise and the associated flooding of mangrove forests, as well as the drying up of individual mangrove ecotopes (FAO 2007; Simard et al. 2019). Commented [AM1]: I suggest improving the English of the

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Thus, the study of mangrove forests is of undoubted interest to scientists. One of the main directions of such research is research related to the study of the physiological characteristics of mangroves. The most significant of these are studies related to their gas exchange (Clough 1997). Nevertheless, despite sufficient knowledge in this area of research, many questions remain in the shadows. In particular, this applies to the mangroves of Vietnam.

55 According to our estimates, one of the most common tree species in the mangrove forests of 56 57 South Vietnam is Rhizophora apiculata Blume, 1827. This species is widely used in reforestation activities (Hogarth 2007). Forming grandiose plantations due to its stilted roots, R. apiculata plays 58 an important role in the ecology of mangrove forests (Thongjoo et al. 2018; Wenfang et al. 2020). 59 Of undoubted interest is the fact that R. apiculata belongs to plants with C4 photosynthesis, which 60 allows the plant to better adapt when growing in conditions of high temperatures and lack of water 61 (Ehleringer and Björkman 1977; Slack and Hatch 1967). Therefore, it is not accidental that many 62 researchers pay attention to this species (Christensen 1978; Ong et al. 1995).

63 Our previous studies carried out on mature R. apiculata trees showed that photosynthesis 64 depression in these trees began to manifest itself at noon and was observed until the end of daylight 65 hours (Đỗ Phong Lưu et al. 2021). In accordance with this, we put forward a hypothesis that the 66 parameters characterizing the photosynthetic abilities of R. apiculata should differ at different times 67 of the day. We did not find any studies confirming or refuting our assumption. We also assumed that 68 the indicated parameters should also differ in plants growing in different conditions. 69

In accordance with the hypothesis put forward, the purpose of the work was determined: to study the daily variability of the parameters of the photosynthetic ability of R. apiculata seedlings of artificial and natural origin.

70 71 72 73 In accordance with the purpose of the work, the following tasks were set: to obtain daily dynamics of the intensity of photosynthesis for seedlings of artificial and natural origin, to model the 74 response curves of photosynthesis to light according to the Michaelis-Munten equation (1), to obtain the dependence of photosynthesis on temperature and  $CO_2$  concentration in the air, to conduct analysis of the obtained results.

75 76 77 This article presents the results of our research related to the study of the photosynthetic exchange 78 of CO2 at the leaf level in-situ of seedlings of R. apiculata of natural and artificial origin. These results 79 can be used to recalculate photosynthesis at the leaf level, down to the planting level. Also, the results 80 obtained will contribute to predicting the growth of plants of this species both in the current period 81 of time and in the future in connection with global climatic changes. In practice, the research results 82 will be useful in the development of reforestation measures. 83

#### MATERIALS AND METHODS

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Study site, plant material and growing conditions

86 The research was carried out in July 2020 in a mangrove plantation located in the Can Gio 87 Biosphere Reserve, located 50 km from Ho Chi Minh City (10°28'36"N, 106°54'17"E) (South 88 Vietnam). Seedlings of Rhizophora apiculata Blume, 1827, about 5 years old, of artificial and natural 89 origin, were chosen as the test material.

90 Artificial seedlings grew in an open area and were intended for reforestation activities (Fig. 1a). 91 The number of studied seedlings n = 27, their average height h = 57 cm (Standard Deviation, SD = 792 cm), the average number of leaves per seedling N = 35 (SD = 16).

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Fig. 1. Studied seedlings of *R. apiculata* of artificial (a) and natural (b) origins

Natural seedlings grew along the edge of the water channel on its northern side (Fig. 1b). The seedlings were formed as a result of the germination of floating fruits that were washed ashore. At noon, the seedlings were shaded by the trees and shrubs growing behind them. Seedling parameters: n = 14, h = 88 cm (SD = 8 cm), N = 10 (SD = 5).

Twice a day, both sites were flooded with water as a result of sea tides.

#### Measurement of photosynthetic gas exchange and experimental design

Photosynthesis processes were considered from the standpoint of CO<sub>2</sub> gas exchange. The rate of photosynthesis (photosynthesis) was measured using a Portable Photosynthesis System LI-6800 (Li-Cor Inc., USA). For artificial illumination of the investigated part of the sheet, a  $3 \times 3$  cm light source was used, supplied by the LI-6800 manufacturer as an addition to the device. The emission spectrum of the light source consists of red ( $\lambda = 660$  nm) and blue ( $\lambda = 453$  nm) colors. When using it, the object was illuminated with light, consisting of red and blue colors in a ratio of 9: 1. During measurements under natural light, photosynthetically active radiation (PAR) was measured using a sensor located in the LI-6800 measuring chamber.

During measurements, the required microclimate parameters were set in the LI-6800 measuring chamber - object illumination, air temperature and humidity, and CO<sub>2</sub> concentration.

For the study, we used the formed intact leaves, as a rule, located on the penultimate node of the shoot. The measurements were carried out in the middle part of the leaf, bounded by the frame of the LI-6800 measuring chamber with an aperture of 3x3 cm. Current measurements of photosynthesis were carried out on 2-4 randomly selected seedlings. To construct the diurnal graphical dependencies, the average values of the measured values were used.

Studies of the dependence of photosynthesis on temperature were carried out on artificial seedlings. During measurements, the following microclimate parameters were set in the LI-6800 measuring chamber: illumination  $1000 \,\mu mol \cdot m^{-2} \cdot s^{-1}$ , CO<sub>2</sub> concentration  $400 \,\mu mol \cdot mol^{-1}$ , humidity ~ 60%. The measurements were carried out in an automatic mode in the temperature range from 24 to 46 °C.

122 Studies of the dependence of photosynthesis on  $CO_2$  concentration were also carried out on 123 artificial seedlings. During measurements, the following microclimate parameters were set in the LI-124 6800 measuring chamber: illumination 1000  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>, temperature 30 °C, humidity ~ 70%. The 125 measurements were carried out in an automatic mode in the CO<sub>2</sub> concentration range from 350 to 126 1000  $\mu$ mol·mol<sup>-1</sup> with a step of 50  $\mu$ mol·mol<sup>-1</sup>. The work used meteorological data obtained from a meteorological station located on the territory of the reserve from a meteorological station located on the reserve's territory.

#### Diurnal curves of photosynthesis and PAR

131 The data for plotting the diurnal dynamics of photosynthesis and PAR were obtained over two 132 days: July 4, 2020, from 15:00 to 19:00 and on July 27, 2020, from 05:00 to 14:30. The total solar 133 radiation these days differed by 6% (the cloudiness on July 4, 2020, in the first half of the day was 134 slightly higher). The average air temperature during the daylight hours on July 4, 2020, was 34 °C, 135 on July 27, 2020 - 36 °C.
136 The measurements were carried out with an interval of ~20 min with the following microclimate

The measurements were carried out with an interval of ~20 min with the following microclimate
parameters in the LI-6800 measuring chamber: CO<sub>2</sub> content 400 μmol·mol<sup>-1</sup>, humidity ~65%,
temperature ~32 °C.

#### Light-response curves

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The mathematical description of the curves of the light response of photosynthesis was based on
 the Michaelis - Munten equation (Michaelis and Muenten 1913). We used this equation in a modified
 form (Kaipiainen 2009):

#### $A = A_{\rm m} \cdot Q/(Q + K_{\rm M}) + A_{\rm d} (1),$

145 where A is the intensity of photosynthesis,  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>;  $A_m$  - is the maximum intensity of 146 photosynthesis,  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>;  $A_d$  - respiration rate at Q = 0,  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>;  $K_M$ 147 - Michaelis constant ( $K_M$  is numerically equal to PAR, at which the intensity of photosynthesis is half 148 of the maximum  $A = 0.5A_m$ ). The values of this constant are often used by researchers when 149 comparing the physiological characteristics of plants (Hieke et al. 2002). According to (1), the light 150 compensation point (*LCP*) was determined,  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>, which shows at what intensity of PAR 151 photosynthesis becomes zero.

To assess the efficiency of photosynthesis, we propose to use the slope of the tangent *a* (italic font) to the function curve of (1) at the point corresponding to *K*<sub>M</sub>. From a physical point of view, this coefficient reflects the rate of change in the intensity of photosynthesis with a change in PAR by one unit.

#### Statistical analysis

158 Data processing was carried out using the MS Excel "Descriptive statistics" package (p < 0.05). 159 The degrees of association of the studied datasets were determined using Pearson's correlation 160 coefficients, k. The parameters of equation (1) were selected using the MS Excel package "Parameters 161 of the solution search" (the limiting number of iterations is 100, the relative error is 0.00001, the 162 permissible deviation is 5%, the convergence is 0.0001). The slope a of the tangent, the coefficients 163 of the equation for this tangent, as well as the extremum points of the graphical dependencies were 164 determined using differentiation methods. The total values of the investigated quantities were 165 determined by the integration method. Graphing was carried out using the MS Excel environment. 166

#### RESULTS

168 Diurnal dynamics of photosynthesis and PAR

Fig. 2 shows the daily dynamics of the intensity of photosynthesis and PAR, obtained as a result of measurements on seedlings of artificial origin.



# Fig. 2. Daily dynamics of the intensity of photosynthesis -A, and PAR -Q, of artificial seedlings

The dependences of photosynthesis and PAR for these seedlings are characterized by a good degree of the association during the day, k = 0.78. On the other hand, when analyzing the degree of association of these quantities in the pre-noon (from 06:00 to 09:30), noon (from 09:30 to 15:30), and afternoon (from 15:30 to 18:30), the following k values were obtained: 0.85, 0.50 and 0.87, respectively.

The total PAR for artificial seedlings was  $46.9 \pm 2.4 \text{ mol} \cdot \text{m}^{-2}$ . In the first half of the day (up to ~ 12 h), it was  $24.2 \pm 1.3 \text{ mol} \cdot \text{m}^{-2}$ , in the second -  $22.8 \pm 1.2 \text{ mol} \cdot \text{m}^{-2}$ .

The total CO<sub>2</sub> exchange for these seedlings was  $0.304 \pm 0.016 \text{ mol}\cdot\text{m}^{-2}$ . However, both in the first half of the day (up to ~ 12 h) and in the second, it was the same and amounted to  $0.154 \pm 0.008$  and  $0.151 \pm 0.008 \text{ mol}\cdot\text{m}^{-2}$ , respectively.

Fig. 3 shows the daily dynamics of the intensity of photosynthesis and PAR, obtained as a result of measurements on seedlings of natural origin.



Fig. 3. Daily dynamics of the intensity of photosynthesis - A, and PAR - Q, seedlings of natural origin

190 The dependences of photosynthesis and PAR for these seedlings are also characterized by a good 191 degree of the association during the day, k = 0.78. When analyzing the degree of association of these 192 dependencies in the pre-noon, noon, and afternoon hours, the following k values were obtained: 0.96, 193 0.77, and 0.90, respectively.

194 The total PAR for seedlings of natural origin was  $24.2 \pm 1.3 \text{ mol} \cdot \text{m}^{-2}$ . In the first half of the day 195 (up to ~ 12 h), it was  $5.8 \pm 0.3 \text{ mol} \cdot \text{m}^{-2}$ , in the second -  $18.4 \pm 1.0 \text{ mol} \cdot \text{m}^{-2}$ .

The total CO<sub>2</sub> exchange for these seedlings was  $0.241 \pm 0.013 \text{ mol} \cdot \text{m}^{-2}$ . In the first half of the day (up to ~12 h), it was  $0.103 \pm 0.006$ , in the second -  $0.138 \pm 0.007 \text{ mol} \cdot \text{m}^{-2}$ .

Light-response curves

200 Figure 4 shows the values of photosynthesis depending on PAR, measured on seedlings of 201 artificial (Fig. 4a) and natural (Fig. 4b) origin. The figures also show the curves approximating these 202 values, constructed according to (1) for the values obtained in the pre-noon (curves 1) and in the 203 afternoon (curves 2) and tangents to these curves at the points corresponding to the  $K_M$  values.

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Photosynthesis values obtained in the pre-noon time are indicated by markers in the form of circles, at noon - in the form of triangles, in the afternoon - in the form of open circles. 205



Fig. 4. Dependences of the intensity of photosynthesis - A, on PAR - Q, obtained on seedlings of artificial (a) and natural (b) origins. These figures also show the curves approximating these values, constructed according to (Equation 1) for the values obtained in the pre-noon (curves 1) and in the afternoon (curves 2) and tangents to these curves at the points corresponding to the K<sub>M</sub> values

The indicators characterizing the photosynthetic characteristics of seedlings obtained according to (1), as well as the  $R^2$  values for the curves plotted and the number of measurements n, are summarized in Table 1.

Seedlings of artificial origin Seedlings of natural origin Index Pre-noon time Afternoon time Pre-noon time Afternoon time  $R^2$ 0.97 0,90 0.95 0,89 п 124 94 Am, 9.5 13.5 8.9 16.0  $\mu mol \cdot m^{-2} \cdot s^{-1}$ 0.013 0.006 0.012 0.007 а 202.5 202.7 204.2 204.6 Kм  $A_{d}$ , -1.5 -1.0  $\mu mol \cdot m^{-2} \cdot s^{-1}$ LCP, 25.9 21.0 38.0 16.3 µmol·m<sup>-2</sup>·s<sup>-1</sup>

#### Table 1. Indicators characterizing photosynthetic characteristics of seedlings.

Dependence of photosynthesis on temperature and CO<sub>2</sub> concentration

The obtained values of the intensity of photosynthesis for seedlings of artificial origin, as a function of the temperature T of the air surrounding the leaf, are approximated by a quadratic equation (R<sup>2</sup>=0.97, n=20):

 $A(T) = -0.0889T^2 + 6.2453T - 100.84 \ (2).$ 

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The extremum of this function corresponds to a value of T = 35 °C. Therefore, taking into account measurement errors, the optimal temperature for photosynthesis of *R. apiculata* seedlings is  $T_{opt} =$ (35 ± 2) °C. The dependence of the intensity of photosynthesis of seedlings on the concentration of *CO*<sub>2</sub> in

The dependence of the intensity of photosynthesis of seedlings on the concentration of  $CO_2$  in the air is described by a linear equation ( $R^2$ =0.95, n=13):

 $A(CO_2) = 0.0056CO_2 + 2.62$  (3).

#### DISCUSSION

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Effect of PAR on photosynthesis

The diurnal dynamics of the intensity of photosynthesis of seedlings of artificial origin (Fig. 2) show the following distinctive patterns:

1) an increase in photosynthesis to maximum values in the pre-noon time, up to 09:30, in proportion to an increase in PAR (k = 0.85);

2) a decline in photosynthesis from maximum values to values corresponding on average to 8.0  $\mu$ mol·m<sup>2</sup>·s<sup>-1</sup> (SD = 2.4  $\mu$ mol·m<sup>2</sup>·s<sup>-1</sup>), and the exit of the photosynthesis curve to a kind of plateau at noon, from 09:30 to 15:30, with a weak dependence of photosynthesis on PAR (k = 0.50);

3) a decline in photosynthesis in the afternoon, from 15:30, in proportion to a decrease in PAR (k = 0.87);

4) negative values, indicating the processes of respiration occurring in the leaf, at night.

It is interesting to note that the same diurnal dynamic was obtained for the light leaves of *Rhizophora mucronata* Poir. growing in Indian red mangroves (Kumar et al. 2017).

In order to give a more detailed interpretation of the listed patterns, let us turn to the dependences
 of photosynthesis on PAR (Fig. 4a) and indicators characterizing the photosynthetic characteristics
 of seedlings (Table 1).

As can be seen from Fig. 4a, the markers representing the obtained photosynthesis values are distributed in three clearly marked zones: 1 - in the zone corresponding to the photosynthesis values obtained in the pre-noon (markers in the form of circles); 2 - in the zone corresponding to the values obtained at noontime (triangular markers); 3 - in the zone corresponding to the values obtained in the afternoon (markers in the form of open circles).

The curves plotted according to (1) for the photosynthesis values obtained in the pre-noon and afternoon (Fig. 4a) have a high degree of association (Table 1). The  $K_{\rm M}$  coefficients characterizing the physiological characteristics of plants are approximately the same, which is obvious since we examined plants of the same type. However, the maximum intensity of photosynthesis,  $A_{\rm m}$ , in seedlings in the pre-noon time was significantly higher than in the afternoon. The same applies to the slopes *a*. Thus, in artificial seedlings, the efficiency of photosynthesis in the pre-noon time was 2.2 times higher than in the afternoon.

260 On the other hand, the *LCP* for these seedlings in the afternoon was 1.8 times higher than that in 261 the pre-noon. That is, in the afternoon, at a PAR of  $38.0 \ \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , the absorption of CO<sub>2</sub> by the 262 leaf was compensated by its release. Such processes are caused by respiration, as a rule, associated 263 with metabolic processes occurring in the leaf.

264 In addition to the above, it can be noted that, according to Figures 2 and 4a, the saturation of 265 photosynthesis for these seedlings occurs when the PAR is about  $1800 \,\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

A similar pattern in the dynamics of photosynthesis, indicating its different behavior in the prenoon and afternoon, was not identified by the authors of this work in similar studies. However, it should be noted that the authors of the work (Ball et al. 1997), when conducting similar studies, noted a very large scatter of data. In our studies, with the exception of midday measurements, this was not noted.

The daily dynamics of photosynthesis in seedlings of natural origin (Fig. 3), in general, is characterized by the same regularities as in seedlings of artificial origin. Distinctive features of this Commented [AM5]: Besides the interpretation of findings, I suggest the discussion part might address the comparison of your result to previous studies and update the literature review that validates your findings. Moreover, at the end of this part, conclude your study and the significance of your research. 273 dynamics are somewhat large values of *k* obtained before and afternoon (0.96 and 0.90), as well as 274 the presence of a relationship between photosynthesis and PAR at noon (k = 0.77).

A similar situation emerges when analyzing the features of photosynthesis of seedlings according
 to Figure 4b - the obtained values of photosynthesis are also distributed in the three zones indicated
 above.

The plotted curves (1) for the values of photosynthesis obtained before and afternoon also have a high degree of association (Table 1). The  $K_{\rm M}$  coefficients are approximately the same. The maximum intensity of photosynthesis,  $A_{\rm m}$ , in seedlings in the pre-noon time is significantly higher than in the afternoon. The same applies to the slopes *a*. Thus, in seedlings of natural origin, the efficiency of photosynthesis in the pre-noon time was 1.7 times higher than in the afternoon. The *LCP* for these seedlings in the afternoon was 1.6 times higher than that in the pre-noon time. In the afternoon, *LCP* was 25.9  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>.

Summarizing what has been said, we can present a comparative analysis of the characteristics ofthe growth of seedlings of artificial and natural origin.

287 On the one hand, these seedlings have similar characteristics. Thus, the diurnal dynamics of 288 seedlings is characterized by an increase in photosynthesis in the pre-noon and a decrease in 289 photosynthesis in the afternoon, with strong degrees of connection with PAR (Fig. 2, 3). The obtained 290 values of photosynthesis are distributed in three clearly marked zones, corresponding to the values 291 of photosynthesis obtained in the pre-noon, noon and afternoon (Fig. 4a, 4b). Photosynthesis values 292 obtained in the pre and afternoon time are described with a high degree of association (1).

Slope coefficients *a* obtained for seedlings during pre-noon and during the afternoon are identical (Table 1). Accordingly, the efficiency of photosynthesis during pre-noon and during the afternoon is approximately the same and during pre-noon, it is higher than during the afternoon. *K*<sub>M</sub> coefficients for seedlings are approximately the same. *LCP* for seedlings in the afternoon was higher than that during pre-noon.

298 On the other hand, the seedlings under consideration also have distinctive characteristics. So, at 299 noon, photosynthesis in seedlings of artificial origin was more stochastic, while in seedlings of natural 300 origin, there is a connection between photosynthesis and PAR. Further, the maximum values of 301 photosynthesis,  $A_{\rm m}$ , for seedlings of artificial origin, both during pre-noon and afternoon, were 302 significantly higher than those of seedlings of natural origin. This is primarily due to different lighting 303 conditions of seedlings: the total PAR for artificial seedlings was  $46.9 \pm 2.4 \text{ mol}\cdot\text{m}^{-2}$  (in the first and 304 second half of the day it was on  $23.5 \pm 1.3 \text{ mol} \cdot \text{m}^{-2}$ ), while for seedlings natural origin -  $24.2 \pm 1.3$ 305 mol·m<sup>-2</sup> (in the first half of the day it was  $5.8 \pm 0.3$  mol·m<sup>-2</sup>, in the second -  $18.4 \pm 1.0$  mol·m<sup>-2</sup>).

306 Accordingly, the total CO<sub>2</sub> gas exchange for artificial seedlings was  $0.304 \pm 0.016 \text{ mol}\cdot\text{m}^{-2}$  (in 307 the first and second half of the day it was the same and amounted to on  $0.153 \pm 0.008 \text{ mol} \text{ m}^{-2}$ ), 308 whereas, for seedlings of natural origin, the total CO2 exchange was  $0.241 \pm 0.013 \text{ mol}\cdot\text{m}^{-2}$  (in the 309 first half of the day it was  $0.103 \pm 0.006$ , in the second -  $0.138 \pm 0.007 \text{ mol}\cdot\text{m}^{-2}$ ). 310 The saturation of photosynthesis for these seedlings (Fig. 3 and 4b) occurs when PAR equals

The saturation of photosynthesis for these seedlings (Fig. 3 and 4b) occurs when PAR equals about 1500  $\mu$ mol·m<sup>2</sup>·s<sup>-1</sup>.

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312 It should be noted here that the saturation values of photosynthesis obtained by us, both for 313 artificial seedlings and for seedlings of natural origin, are fundamentally different from those 314 presented in (Ball et al. 1997) that was amounting to about 400  $\mu$ mol m<sup>-2</sup>·s<sup>-1</sup>.

Thus, seedlings of artificial origin had higher productivity, and this was determined, first of all, by the amount of PAR supplied to the plants. Higher metabolic processes occurring in the leaves of plants of seedlings of artificial origin are also indicated by higher values of *LCP* and  $A_d$  (Table 1).

318Naturally, the considered physiological parameters affected the morphological characteristics of319the seedlings. Thus, the average number of leaves on a seedling of artificial origin is N = 35 (SD =32016), while on a seedling of natural origin - N = 10 (SD = 5). However, such a significant difference321in the number of leaves on seedlings was to some extent compensated by the height - seedlings of322natural origin were 1.5 times higher.

#### Effect of temperature and CO<sub>2</sub> concentration on photosynthesis

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325 Analysis (2) showed that the optimal air temperature for photosynthesis of R. apiculata is  $T_{opt} = (35)$ 326 ± 2) °C. It can be noted here that our similar studies on an adult tree R. apiculata of natural origin 327 gave the same result (Đỗ Phong Lưu et al. 2021). According to (Ball et al. 1997), this figure is 328 approximately 38 °C. However, the authors noted a large scatter of data and, unfortunately, do not 329 indicate the amount of error in the determined value. In another work (Okimoto et al. 2013), two 330 values of this temperature are given: 33 °C and 26 °C. Because the authors used similar equipment 331 to carry out their studies, taking into account the error, we can say that the first temperature 332 coincides with  $T_{opt}$ . Thus, the deviation of the air temperature from  $T_{opt}$ , both to a lower and to a 333 higher side, causes a decrease in photosynthesis in R. apiculata (Sage and Kubien 2007).

The average air temperature during measurements at noon was 37.2 °C (SD = 1.0 °C). However, such temperatures could cause a decrease in photosynthesis by only 0.1%. On the other hand, we did not measure the temperature of the leaves, which, as a result of exposure to direct solar radiation (Fig. 1a and 2), could be quite high. High leaf temperatures inhibit photosynthesis. In addition, plants could experience a water shortage. For example, our studies related to the moisture content of leaves in relation to their absolutely dry weight on an adult *R. apiculata* tree showed that from 08:50 to 15:20 the leaves were losing 34% of moisture.

341 It is possible that in different leaves of seedlings, water deficiency manifested itself in different 342 ways with corresponding changes in photosynthesis. At least, this hypothesis can explain the 343 stochastic distribution of the values of photosynthesis in artificial seedlings at noon.

In contrast to this, in seedlings of natural origin, the presence of a connection between photosynthesis and PAR was noted at noon. This is due to the fact that these seedlings in the midday time were shadowed by the trees and shrubs growing behind them (Fig. 1b and 3). As a result, the leaves of these seedlings were exposed to significantly less overheating and so experienced less water deficit.

Based on what has been said, we can make the following assumption. In the studied seedlings,
the water consumed during midday time was not completely restored afterwards. This can explain
the significantly lower photosynthetic parameters observed in seedlings during afternoon (Table 1)
than during pre-noon. This assumption is confirmed by the conclusions made in the work (Kumar et
al. 2017).

The dependence of the intensity of photosynthesis of *R. apiculata* on the concentration of CO<sub>2</sub> in the air is described by (3). It follows from this equation that with an increase in CO<sub>2</sub> concentration, photosynthesis naturally increases. An increase in the growth of *R. apiculata* seedlings at increased CO<sub>2</sub> concentration in the air is noted in in (Eong et al. 1997; Kumar et al. 2017).

358 The presented dependences of photosynthesis on temperature and CO<sub>2</sub> concentration find their 359 confirmation also in a number of works related to the study of the effect of elevated temperature and 360 CO<sub>2</sub> concentration, simulating global warming, on photosynthesis of C4 plants (Alberto et al. 1996; 361 Ghannoum et al. 2000; Read and Morgan 1996; Morgan et al. 1994). In our case, for example, if we 362 consider the most optimistic forecasts associated with an increase in the concentration of CO<sub>2</sub> in the 363 air in the next decade from 412 ppm to 460 ppm (and this concentration is already observed over 364 cities), then the intensity of photosynthesis in the studied seedlings will increase by about 6%. This 365 trend will be one of the tools for stabilizing the climate on Earth.

#### CONCLUSIONS

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1. The determining factor influencing the photosynthesis of the investigated seedlings of *R*.
 *apiculata* is PAR. This determines the higher productivity of artificial seedlings in comparison with
 natural seedlings.

3712. The obtained photosynthesis values are distributed in three clearly marked zones,372 corresponding to the photosynthetic values obtained in the pre-noon, noon and afternoon.

Commented [AM6]: I suggest the conclusions offer a clear interpretation of the findings in a way that emphasizes the importance of your study or describes the consequences of your arguments by justifying to your readers why your arguments matter. A conclusion must be broader and more comprehensive than specific or limited findings, and in the same vein, several findings may be combined into a single conclusion.

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3. In the studied seedlings, the water consumed in the midday time was not completely restored
afterwards. As a result, the photosynthetic parameters of the seedlings in the pre-noon were
significantly higher than in the afternoon.
4. The main inhibiting factor affecting the photosynthesis of *R. apiculata* (if we do not take into

4. The main inhibiting factor affecting the photosynthesis of *R. apiculata* (if we do not take into account PAR) is the disturbance of the water balance of the leaves (lack of water).

5. The optimum air temperature for the processes of photosynthesis in *R. apiculata* seedlings is  $(35 \pm 2)$  °C.

6. With an increase in the concentration of  $CO_2$  in the air, the intensity of photosynthesis in *R*. *apiculata* naturally increases.

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